

CLAIMS

1. Method for purifying recombinant HCV single or specific oligomeric envelope proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulphide bond cleavage or reduction step is carried out with a disulphide bond cleavage agent.
2. Method according to claim 1, wherein said disulphide cleavage or reduction step is carried out under partial cleavage or reducing conditions.
3. Method according to claim 1 or 2, wherein said disulphide bond cleavage agent is dithiothreitol (DTT), preferably in a concentration range of 0.1 to 50 mM, preferably 0.1 to 20 mM, more preferably 0.5 to 10 mM.
4. Method according to claim 1, wherein said disulphide bond cleavage agent is a detergent.
5. Method according to claim 4, wherein said detergent is Empigen-BB, preferably at a concentration of 1 to 10%, more preferably at a concentration of 3.5%.
6. Method according to claim 1 or 2, wherein said disulphide bond cleaving agent comprises a combination of a classical disulphide bond cleavage agent, such as DTT, and a detergent, such as Empigen-BB.
7. Method according to any of claims 1 to 6, further comprising the step of blocking disulphide bond reformation with an SH group blocking agent.
8. Method according to claim 7, wherein said SH group blocking agent is N-ethylmaleimide (NEM) or a derivative thereof.
9. Method according to claim 7, wherein said step of blocking the disulphide bond reformation is brought about by low pH conditions.

10. Method according to any of claims 1 to 9, further characterized by at least the following steps:

- lysing recombinant E1 and/or E2 and/or E1/E2 expressing host cells, possibly in the presence of an SH blocking agent such as N-ethylmaleimide (NEM),
- recovering said HCV envelope proteins by affinity purification such as by means of lectin-chromatography, such as lentil-lectin chromatography, or by means of immunoaffinity using anti-E1 and/or anti-E2 specific monoclonal antibodies,
- reduction or cleavage of the disulfide bonds with a disulphide bond cleaving agent, such as DTT, preferably also in the presence of an SH blocking agent, such as NEM or Biotin-NEM, and,
- recovering the reduced E1 and/or E2 and/or E1/E2 envelope proteins by gel-filtration and possibly also by a subsequent Ni-IMAC chromatography and desalting step.

11. Composition comprising essentially purified recombinant HCV single or specific oligomeric recombinant envelope proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized as being isolated by a method according to any of claims 1 to 10.

12. Composition according to claim 11, further characterized in that said recombinant HCV envelope proteins are expressed from recombinant mammalian cells such as vaccinia.

13. Composition according to claim 11, further characterized in that said recombinant HCV envelope proteins are expressed from recombinant yeast cells.

14. Composition according to claim 11, further characterized in that said recombinant HCV envelope proteins are the expression product of at least one of the recombinant vectors according to any of claims 15 to 24.

15. Recombinant vector comprising a vector sequence, an appropriate prokaryotic, eukaryotic or viral promoter sequence followed by a nucleotide sequence allowing the expression of a single or specific oligomeric E1 and/or E2 and/or E1/E2 protein.

16. Recombinant vector according to claim 15, with said nucleotide sequence being characterized further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 1 and 192 and ending in the region between amino acid positions 250 and 400, more particularly ending in the region between positions 250 and 341, even more preferably ending in the region between position 290 and 341.

17. Recombinant vector according to claim 16, with said nucleotide sequence being characterized further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 117 and 192 and ending in the region between amino acid positions 263 and 400, more particularly ending in the region between positions 250 and 326.

18. Recombinant vector according to any of claims 16 or 17, with said nucleotide sequence being characterized further in that it encodes a single HCV E1 protein bearing a deletion of the first hydrophobic domain between positions 264 to 293, plus or minus 8 amino acids.

19. Recombinant vector according to claim 15, with said nucleotide sequence being characterized further in that it encodes a single HCV E2 protein starting in the region between amino acid positions 290 and 406 and ending in the region between amino acid positions 600 and 820, more particularly starting in the region between positions 322 and 406, even more preferably starting in the region between position 347 and 406 and most preferably starting in the region between positions 364 and 406.

20. Recombinant vector according to claim 19, with said nucleotide sequence being characterized further in that it ends at any of amino acid positions 623, 650, 661, 673, 710, 715, 720, 746 or 809.

21. Recombinant vector according to any of claims 16 to 20, with said nucleotide sequence being characterized further in that a 5'-terminal ATG codon and a 3'-terminal stop codon have been added to it.

22. Recombinant vector according to any of claims 16 to 21, with said nucleotide

sequence being characterized further in that a factor Xa cleavage site and/or 3 to 10, preferably 6, histidine codons have been added 3'-terminally to the coding region.

23. Nucleic acid comprising any of the sequences as represented in SEQ ID NO 3, 5, 7, 9, 11, 13, 21, 23, 25, 27, 29, 31, 35, 37, 39, 41, 43, 45, 47 and 49, or parts thereof.

24. Recombinant vector carrying a recombinant nucleic acid according to claim 23.

25. Recombinant vector according to any of claims 15 to 24, further characterized in that at least one of the glycosylation sites present in said E1 or E2 protein has been removed at the nucleic acid level.

26. A host cell transformed with at least one recombinant vector according to any of claims 15 to 26, wherein said vector comprises a nucleotide sequence encoding HCV E1 and/or E2 and/or E1/E2 protein as defined in any of claims 15 to 23 in addition to a regulatory sequence operable in said host cell and capable of regulating expression of said HCV E1 and/or E2 and/or E1/E2 protein.

27. A recombinant E1 and/or E2 and/or E1/E2 protein expressed by a host cell according to claim 26.

28. Method according to any of claims 1 to 10, further characterized as comprising at least the following steps:

- growing a host cell as defined in claim 26 transformed with a recombinant vector according to any of claims 15 to 25 in a suitable culture medium,
- causing expression of said vector sequence as defined in any of claims 16 to 25 under suitable conditions, and,
- lysing said transformed host cells, preferably in the presence of an SH group blocking agent, such as N-ethylmaleimide (NEM),
- recovering said HCV envelope protein by affinity purification by means of for instance lectin-chromatography or immunoaffinity chromatography using anti-E1 and/or anti-E2 specific monoclonal antibodies, with said lectin being preferably lentil-lectin, followed by,
- incubation of the eluate of the previous step with a disulphide bond cleavage

agent, such as DTT, preferably also in the presence of an SH group blocking agent, such as NEM or Biotin-NEM, and,

isolating the HCV single or specific oligomeric E1 and/or E2 and/or E1/E2 proteins by means of gelfiltration and possibly also by means of an additional Ni^{2+} -IMAC chromatography and desalting step.

29. A composition comprising at least one of the following E1 and/or E2 peptides:

E1-31 (SEQ ID NO 56) spanning amino acids 181 to 200 of the Core E1 V1 region,

E1-33 (SEQ ID NO 57) spanning amino acids 193 to 212 of the E1 region,

E1-35 (SEQ ID NO 58) spanning amino acids 205 to 224 of the E1 V2 region (epitope B),

E1-35A (SEQ ID NO 59) spanning amino acids 208 to 227 of the E1 V2 region (epitope B),

1bE1 (SEQ ID NO 53) spanning amino acids 192 to 228 of E1 regions (V1, C1, and V2 regions (containing epitope B),

E1-51 (SEQ ID NO 66) spanning amino acids 301 to 320 of the E1 region,

E1-53 (SEQ ID NO 67) spanning amino acids 313 to 332 of the E1 C4 region (epitope A),

E1-55 (SEQ ID NO 68) spanning amino acids 325 to 344 of the E1 region.

Env 67 or E2-67 (SEQ ID NO 72) spanning amino acid positions 397 to 416 of the E2 region (epitope A),

Env 69 or E2-69 (SEQ ID NO 73) spanning amino acid positions 409 to 428 of the E2 region (epitope A),

Env 23 or E2-23 (SEQ ID NO 86) spanning positions 583 to 602 of the E2 region (epitope E),

Env 25 or E2-25 (SEQ ID NO 87) spanning positions 595 to 614 of the E2 region (epitope E),

Env 27 or E2-27 (SEQ ID NO 88) spanning positions 607 to 626 of the E2 region (epitope E),

Env 17B or E2-17B (SEQ ID NO 83) spanning positions 547 to 566 of the E2 region (epitope D),

Env 13B or E2-13B (SEQ ID NO 82) spanning positions 523 to 542 of the E2 region (epitope C).

30. A composition comprising at least one of the following E2 conformational epitopes:

epitope F recognized by monoclonal antibodies 15C8C1, 12D11F1, and 8G10D1H9.

5 epitope G recognized by monoclonal antibody 9G3E6.

epitope H (or C) recognized by monoclonal antibodies 10D3C4 and 4H6B2.

epitope I recognized by monoclonal antibody 17F2C2.

31. An E1 and/or E2 specific monoclonal antibody raised upon immunization with a composition according to any of claims 11 to 14 or 29 to 30.

10 32. An E1 and/or E2 specific monoclonal antibody according to claim 31 for use as a medicament, more particularly for incorporation into an immunoassay kit for detecting the presence of HCV E1 or E2 antigen, for prognosis/monitoring of disease or for HCV therapy.

15 33. Use of an E1 and/or E2 specific monoclonal antibody according to claim 31 for the preparation of an immunoassay kit for detecting HCV E1 or E2 antigens, for the preparation of a kit for prognosing/monitoring of HCV disease or for the preparation of a HCV medicament.

34. Method for in vitro diagnosis of HCV antigen present in a biological sample, comprising at least the following steps:

- 20 (i) contacting said biological sample with an E1 and/or E2 specific monoclonal antibody according to claim 31, preferably in an immobilized form under appropriate conditions which allow the formation of an immune complex,
- 25 (ii) removing unbound components,
- (iii) incubating the immune complexes formed with heterologous antibodies, with said heterologous antibodies being conjugated to a detectable label under appropriate conditions,
- 30 (iv) detecting the presence of said immune complexes visually or mechanically.

35. Kit for determining the presence of HCV antigens present in a biological sample, comprising:

- at least one E1 and/or E2 specific monoclonal antibody according to claim 31, preferably in an immobilized form on a solid substrate,
- a buffer or components necessary for producing the buffer enabling binding reaction between these antibodies and the HCV antigens present in said biological sample,
- a means for detecting the immune complexes formed in the preceding binding reaction.

36. A composition according to any of claims 11 to 14 or 29 to 30 for use as a medicament.

37. A composition according to any of claims 11 to 14 or 29 to 30 for use as a vaccine for immunizing a mammal, preferably humans, against HCV, comprising administering an effective amount of said composition possibly accompanied by pharmaceutically acceptable adjuvants, to produce an immune response.

38. Use of a composition according to any of claims 11 to 14 or 29 to 30, for the preparation of a vaccine for immunizing a mammal, preferably humans, against HCV, comprising administering an effective amount of said composition possibly accompanied by pharmaceutically acceptable adjuvants, to produce an immune response.

39. Vaccine composition for immunizing a mammal, preferably humans, against HCV, comprising an effective amount of a composition according to any of claims 11 to 14 or 29 to 30 possibly accompanied by pharmaceutically acceptable adjuvants.

40. A composition according to any of claims 11 to 14 or 29 to 30, for *in vitro* detection of HCV antibodies present in a biological sample.

41. Use of a composition according to claims 11 to 14 or 29 to 30, for the preparation of an immunoassay kit for detecting HCV antibodies present in a biological sample.

42. Method for *in vitro* diagnosis of HCV antibodies present in a biological sample, comprising at least the following steps:

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- (i) contacting said biological sample with a composition according to any of claims 11 to 14 or 29 to 30, preferably in an immobilized form under appropriate conditions which allow the formation of an immune complex,
- (ii) removing unbound components,
- 10 (iii) incubating the immune complexes formed with heterologous antibodies, with said heterologous antibodies being conjugated to a detectable label under appropriate conditions,
- (iv) detecting the presence of said immune complexes visually or mechanically.

15 43. Kit for determining the presence of HCV antibodies present in a biological sample, comprising:

- at least one peptide or protein composition according to any of claims 11 to 14 or 29 to 30, preferably in an immobilized form on a solid substrate,
- 20 - a buffer or components necessary for producing the buffer enabling binding reaction between these proteins or peptides and the antibodies against HCV present in said biological sample,
- a means for detecting the immune complexes formed in the preceding binding reaction.

25 44. Use of composition comprising E1 proteins according to any of claims 11 to 14, or parts thereof according to claim 29, more particularly HCV single E1 proteins or E1 peptides, for *in vitro* monitoring HCV disease or prognosing the response to treatment, particularly with interferon, of patients suffering from HCV infection comprising:

- 30 - incubating a biological sample from a patient with HCV infection with an E1 protein or a suitable part thereof under conditions allowing the formation of an immunological complex,
- removing unbound components.

- calculating the anti-E1 titers present in said sample at the start of and during the course of treatment.
- monitoring the natural course of HCV disease, or prognosing the response to treatment of said patient on the basis of the amount anti-E1 titers found in said sample at the start of treatment and/or during the course of treatment.

45. Kit for monitoring HCV disease or prognosing the response to treatment, particularly with interferon, of patients suffering from HCV infection comprising:

- at least one E1 protein or E1 peptide, more particularly an E1 protein or E1 peptide according to any of claims 11 to 14 or 29,
- a buffer or components necessary for producing the buffer enabling the binding reaction between these proteins or peptides and the anti-E1 antibodies present in a biological sample,
- means for detecting the immune complexes formed in the preceding binding reaction,
- possibly also an automated scanning and interpretation device for inferring a decrease of anti-E1 titers during the progression of treatment.

46. A serotyping assay for detecting one or more serological types of HCV present in a biological sample, more particularly for detecting antibodies of the different types of HCV to be detected combined in one assay format, comprising at least the following steps :

- (i) contacting the biological sample to be analyzed for the presence of HCV antibodies of one or more serological types, with at least one of the E1 and/or E2 and/or E1/E2 protein compositions according to any of claims 11 to 14 or at least one of the E1 or E2 peptide compositions according to claim 29, preferentially in an immobilized form under appropriate conditions which allow the formation of an immune complex,
- (ii) removing unbound components,
- (iii) incubating the immune complexes formed with heterologous antibodies, with said heterologous antibodies being conjugated to a detectable label under appropriate conditions.

- (iv) detecting the presence of said immune complexes visually or mechanically (e.g. by means of densitometry, fluorimetry, colorimetry; and inferring the presence of one or more HCV serological types present from the observed binding pattern.

5 47. Kit for serotyping one or more serological types of HCV present in a biological sample, more particularly for detecting the antibodies to these serological types of HCV comprising:

- at least one E1 and/or E2 and/or E1/E2 protein according to any of claims 11 to 14 or E1 or E2 peptide according to claim 29,
- 10 - a buffer or components necessary for producing the buffer enabling the binding reaction between these proteins or peptides and the anti-E1 antibodies present in a biological sample,
- means for detecting the immune complexes formed in the preceding binding reaction,
- 15 - possibly also an automated scanning and interpretation device for detecting the presence of one or more serological types present from the observed binding pattern.

48. A peptide or protein composition according to any of claims 11 to 14 or 29, for immobilization on a solid substrate and incorporation into a reversed phase
20 hybridization assay, preferably for immobilization as parallel lines onto a solid support such as a membrane strip, for determining the presence or the genotype of HCV according to a method of any of claims 42 or 46.

Add B2
Add D5
Add G1